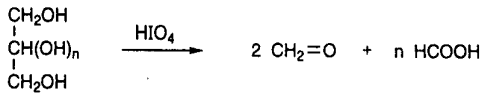
1.3. Reagents Which Split α-Glycolic Linkages

1.3.1. Reaction with Periodic Acid

According to Malaprade[1-3] the C–C bonds of α-glycols are split with periodic acid yielding well-defined carbonyl and carboxyl compounds. Since this reaction is quantitative in many cases, it is applied as a general method for the quantitative determination of vicinal hydroxy compounds. Furthermore, the reaction is also widely used for elucidating the chemical structures of polyhydroxy compounds.

By splitting vicinal polyhydroxy compounds, the periodic acid converts the -CH_2OH group into 1 mole of formaldehyde (HCH=O) and the -CH(OH) group into 1 mole of formic acid (HCOOH) as is demonstrated below using a polyalcohol. The reaction with the *cis*-hydroxyl groups is faster than with those in *trans* position[4,5].



In the case of carbohydrates, two steps of this reaction occur which are named "selective oxidation" and "overoxidation".

In the first step, selective oxidation leads to the formation of formic acid and unique aldehydes, which themselves are not stable since they are hydrolyzed by the formic acid to lower polyhydroxy aldehydes. These are oxidized further by the periodic acid yielding the formic acid and formaldehyde. As an example, the individual steps of the reaction are demonstrated for D-(+)-glucose (see Figure 1)[6].

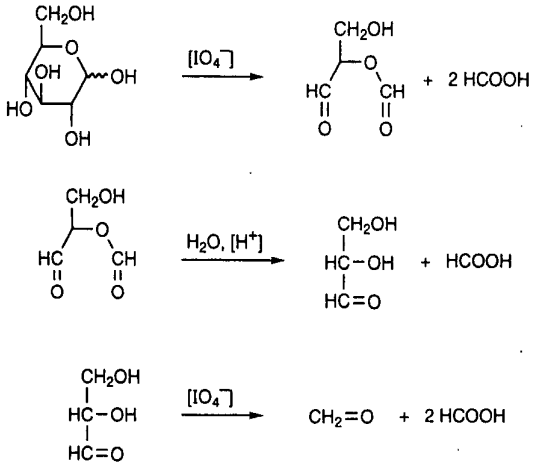


Figure 1. Oxidation of D-Glucose with Periodic Acid (Franzke, Grunert and Obrikat)

The first step involving selective oxidation occurs at high speed. One mol D-(+)-glucose react with 3 mol periodic acid to give cleavage of the C-1–C-2, C-2–C-3 and C-3–C-4 bonds affording 2 moles of formic acid and the formyl derivative of glyceraldehyde. The latter compound would be stable against iodate ions but is hydrolyzed to glyceraldehyde and formic acid; the glyceraldehyde is then again oxidized to a further 2 moles of formic acid and to 1 mole formaldehyde. This second step of overoxidation requires a longer period of time. To summarize, the reaction between D-(+)-glucose and periodic acid yields 5 moles formic acid and 1 mole formaldehyde.

Substitution of the hydrogen of the hydroxyl groups with alkyl residues hinders the attack of periodic acid on the molecules. The same impedance occurs when these hydrogens are substituted by other monosaccharide molecules. The splitting products of the resulting oligosaccharides depend on the location of the substitution.

In some cases overoxidation occurs causing the intermediates of selective oxidation with periodic acid to react further in a so called "non-Malaprade way", e.g. the hydroxylation of active methylene groups and the splitting of enol and of enediol groups[5,7-9] as is demonstrated in the case of the periodic acid oxidation of 1,4-anhydro-D-allitol[10] (see Figure 2).

In order to minimize the overoxidation the following conditions should be observed when the periodic acid oxidation is carried out in practice[11]:

(a) Exclusion of light.

(b) Working within pH 3–5; at this range the optimum splitting of vicinal OH-groups coupled with a minimum of overoxidations has been found.

(c) Performance of the reaction at room temperature.

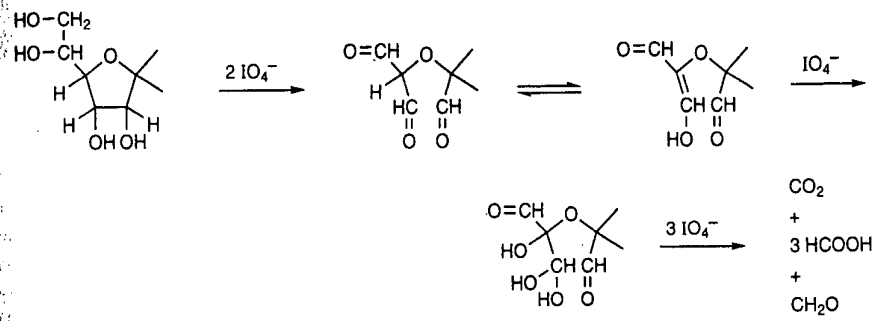


Figure 2. Reaction between 1,4-Dehydro-D-allitol and Periodic Acid (Example of a Non-Malaprade Overoxidation) (Hudson and Barker)

1.3.1.a. Procedure I: Determination of the Yield of Formic Acid and the Consumption of Periodic Acid[11]

Determination

Reagent A: periodic acid (sodium periodate 0.3 M): 45 g sodium periodate are dissolved in 5% aq H_2SO_4. The solution is adjusted to pH 4, initially with 20% NaOH and then with 0.1 mol NaOH (titration against methyl red). Finally, it is made up to 500 mL.

Reagent B: pure ethylene glycol (spectroscopic grade).

Reagent C: sodium arsenite (0.4 M).

Determination of the Formic Acid

Pipet an aliquot part of reagent A to 10 mL of the sample and store the mixture for approximately 4 h; then destroy the excess periodic acid by adding of an excess of ethylene glycol (reagent B; this compound gives only formaldehyde). After a reaction time of 5 minutes titrate the generated formic acid with 0.1 M NaOH against methyl red.

Determination of Periodic Acid Consumption

Pipet an aliquot part of reagent A to 10 mL of the sample and after a distinct reaction time, adjust the mixture with NaOH to pH 8 (titration against phenolphthalein). Then, add 2 g of sodium bicarbonate, an exact volume of reagent C and solid potassium iodide. After standing for 15 min, titrate the excess arsenite with 0.1 M iodine solution.

1.3.1.b. Procedure II: Determination of Microamounts of Monosaccharides[12,13]

The periodate oxidation is carried out in acidic medium and the solution is buffered to a pH of 4.5–7.0. In this medium, the excess of periodic acid reacts with potassium iodide according to the following scheme.

$$IO_4^- + 2I^- + 2H^+ \longrightarrow IO_3^- + I_2 + H_2O$$

The liberated iodine is titrated with sodium thiosulfate. Under these conditions, the further reduction of iodate is kept negligible.

Determination

Reagent A: 1 g of potassium periodate is dissolved in warm water and after cooling made up to 1 L.

Reagent B: 5% H_2SO_4: 2.9 mL of concd H_2SO_4 (σ = 1.84) is diluted with water and made up to 100 mL.

Reagent C: 12% aq solution of *sec*-potassium phosphate.

Pipet to 1 mL of the sample, 8 mL of reagent A and 2 mL of reagent B and then heat the mixture for 20 min in a boiling water bath. After cooling add 4 mL of reagent C and solid potassium iodide and titrate the liberated iodine with 0.005 M sodium thiosulfate against starch. The same procedure is carried out with a blank and different standard solutions. The difference between the volumes of the blank and the standard solutions correlates to the amount of the polyhydroxy compound. By establishing a calibration curve, the amount of the polyhydroxy compound in the sample can be estimated. Complications are observed in the presence of ketoses and oligosaccharides[12]. Aldoses and sugar alcohols consume (n-1) mol periodate and ketoses (n-2) mol, where n is the number of carbon atoms in the sugar molecule.

1.3.1.c. Photometric Procedure for the Determination of Periodate[14]

A photometric method has been developed for the microdetermination of polyhydroxy compounds which works by determining periodic acid consumption.

The periodic acid oxidizes 4-(4-nitrophenoxy)-1,2-butanediol (PND) or 4-(2,4-dinitrophenoxy)-1,2-butanediol (PDB) to the corresponding aldehyde which undergo is β-elimination in alkaline medium giving nitrophenolate with an absorbance at 400 nm (Figure 3).

Determination

Reagent A: aq sodium periodate (0.0518 M).

Reagent B: acetate buffer pH 4.3 (1 M).

Reagent C: aq solution PNB or PDB both (0.013 M).

Reagent D: sodium carbonate–sodium bicarbonate buffer pH 9.5 (0.1 M).

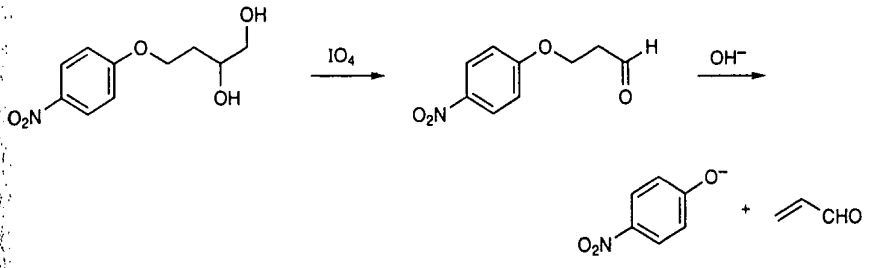


Figure 3. Reaction between Periodate and 4-(4-nitrophenoxy)-1,2-butanediol (Rammler, Bilton, Hangland and Parkinson)

Pipet together 0.1 mL of the sample, 0.2 mL of reagent A and 7.7 mL of reagent B and store the mixture for a distinct time, e.g. 4 h for monosaccharides like D-(+)glucose. Then, add to 0.8 mL of this mixture 0.2 mL reagent C (the amount of PNB or PDB must be in excess) to measure the nonreacted amount of periodic acid. After 15 min, pipet to 0.05 mL of this solution 0.95 mL of reagent D.

After 5 min the absorbance of this solution is read against a control which contains no periodate. All of these reactions are carried out at 50 °C. Parallel to this, the whole reaction is carried out with a blank and with standards of known content of the compound to be determined.

The application of this procedure is described not only for sugars and sugar alcohols but also for nucleosides, nucleotides and ribonucleic acids.

1.3.2. Other Reagents

In addition to periodic acid, a few other reagents split C–C bonds to vicinal hydroxyl groups. To this collection of compounds belongs:

lead(IV) acetate: this reagent can only be applied in nonaqueous medium due to its sensitivity against hydrolysis. The reaction rate is much higher for the *cis*-hydroxyl groups, than for those in the *trans* position[5,15];

active manganese dioxide[16];

potassium peroxodisulfate in presence of silver ions[17];

nickel peroxide[18];

sodium bismuthate(V) [19].

These reagents are widely used in the field of organic synthesis, but not for the determination of polyhydroxy compounds.

References

1 Malaprade, L. *C. R. Acad. Sci.* **1928**, *186*, 382.
2 Malaprade, L. *Bull. Soc. Chim. Fr.* **1928**, *43*, 683.
3 Fleury, P.; Lange, J. *C. R. Acad. Sci.* **1932**, *195*, 1395.
4 Buist, G. J.; Bunton, C. A. *J. Chem. Soc. B* **1971**, 2117, 2128.
5 Perlin, A. S. In Pigman, Horton, Wander (Eds.) *The Carbohydrates I B*; Academic: New York, 1980; p 1167.
6 Barbor, K.; Kalác, V.; Tihlárik, K. *Chem. Zvesti* **1973**, *27*, 676.
7 Schwarz, J. C. F.; McDougall, M. *J. Chem. Soc.* **1956**, 3065.
8 Bose, J. L.; Forster, A. B.; Stephens, R. W. *J. Chem. Soc.* **1959**, 3314.
9 Hesse, G.; Mix, K. *Chem. Ber.* **1959**, *92*, 2427.
10 Hudson, B. G.; Barker, R. *J. Org. Chem.* **1967**, *32*, 2101.
11 Franzke, Cl.; Grunert, K. S.; Obrikat, H. *Z. Lebensm. Unters. Forsch.* **1968**, *136*, 267, 324.
12 Rappaport, E.; Reifer, I.; Weinmann, H. *Mikrochim. Acta* **1937**, (1) 290, (2) 273.
13 Flood, A. E.; Priestley, C. A. *J. Sci. Food Agric.* **1973**, *24*, 945.
14 Rammler, D. H.; Bilton, R.; Haugland, R.; Parkinson, C. *Anal. Biochem.* **1973**, *52*, 198.
15 Criegee, R.; Höger, E.; Huber, G.; Kruck, P.; Marktscheffel, F.; Schellenberger, H. *Liebigs Ann. Chem.* **1956**, *599*, 81.
16 Ohloff, G.; Giersch, W. *Angew. Chem.* **1973**, *85*, 401; *Angew. Chem., Int. ed. Engl.* **1973**, *12*, 401.
17 Greenspan, F. P.; Woodburn, H. M. *J. Am. Chem. Soc.* **1954**, *76*, 6345.
18 Nakagawa, K.; Igano, K.; Sugita, J. *Chem. Pharm. Bull. (Tokyo)* **1964**, *12*, 403.
19 Rigby, W. *J. Chem. Soc.* **1950**, 1907.

1.4. Reaction with Acids

The action of concentrated acids on sugars leads to effective rearrangements due to multiple dehydration steps forming heterocyclic compounds like furfurals and ω-hydroxymethylfurfurals. These compounds react with phenols or aromatic amines giving deep colored substances. In a lot of cases such reactions enable photometric determinations of sugars. Here precise experimental conditions and careful control of the reaction time and acid strength are necessary to obtain reproducible results[1].

1.4.1. General Reactions for Aldoses and Ketoses

The reactions of sugars with phenols and related compounds in the presence of sulfuric acid are universal and positive for the whole group (e.g., aldoses, ketoses, mono-, oligo- and polysaccharides).

1.4.1.1. Reaction with Phenol

Mono-, oligo- and polysaccharides react with phenol and concentrated sulfuric acid at elevated temperatures resulting in the formation of colored substances with absorption maxima at 480–490 nm[1,2]. For the reaction mechanism, it is assumed that, in the case of oligo- and polysaccharides, the external ether bridges are split. Parallel dehydration reactions take place yielding furfural derivatives which condense with phenol to triarylmethane dyes according to the following scheme (see Figure 1).

Pentoses, methylpentoses and hexuronic acids yield orange colored compounds with absorption maxima (λ_{max}) at 490 nm, hexoses and their oligomers such ones with λ_{max} at 490 nm; 2- and 4-ketocarbonic acids interfere with the reaction[3].

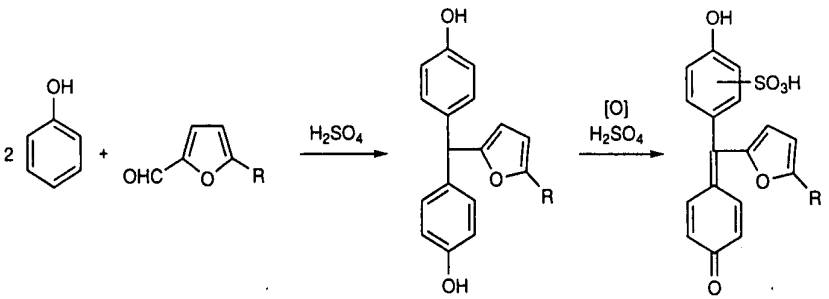


Figure 1. Reaction between Phenol and Carbohydrates in the Presence of Concentrated Sulfuric Acid (Kakác and Vejdelek)

Determination[2,4]

Reagent: mixture of 80 g of cryst phenol with 20 mL of distilled water.

Pipet to 2.0 mL of the aq sample (10–200 μg sugar) 0.05–0.15 mL of the reagent and then carefully add, with intense shaking, 5 mL of concd sulfuric acid. Intensively shake the mixture again and let it stand for 10 min at room temperature and then for 10–20 min in a water bath at 25–30 °C. After this